Examining Partition Efficiency of Cell Types Following QIAcube/EZ1® Advanced XL Differential Extraction

Badiah Hannon[1,2], M.S.; Rachel Houston[1], Ph.D.; Tim Kalafut[1], Ph.D.

[1]Department of Forensics, Sam Houston State University, Huntsville, TX
[2]Houston Forensic Science Center, Houston, TX

INTRODUCTION

Forensic laboratories are challenged with processing the ever-growing backlog of sexual assault kits (SAKs). To combat this backlog, laboratories are encouraged to employ a direct to DNA (DTD) approach, eliminating serology. SAK evidence is processed directly using differential extraction, where two fractions, non-sperm and sperm, are produced. Without serological data, the body fluid contributing the DNA cannot be determined.

In this study, the fractionation of male DNA from various body fluids following differential extraction was examined. The amount of male DNA present in the non-sperm and sperm fractions for each fluid was observed. Based on this data, we were able to determine a difference in fractionation between samples containing sperm and samples not containing sperm.

Enrichment of male DNA in the sperm fraction was also observed using the ratio of total human to male DNA present in the two differential fractions. This data supports the suggestion that sperm behaves differently in differential extraction compared to non-sperm samples. Given this different fractionation pattern, it may be possible to infer the body fluid present in the sperm fraction.

MATERIALS AND METHODS

Sample Collection
Vaginal swabs, male saliva, and semen were collected under verbal consent using Institutional Review Board approved protocols. Single donor male blood was purchased from Innovation Research (Innovative Research Inc, Novi, MI, USA). Each body fluid was deposited onto cotton swabs. Male fluids were deposited onto vaginal swabs and were also applied to jeans and underwear after treatment with a vaginal suspension.

Extraction and Purification
DNA extraction and purification were performed on a QIAcube HID Differential Washing Station (QIAGEN, Hilden, Germany) and EZ1 Advanced XL (QIAGEN) as per the FBI Protocol for STR Analysis (1).

Quantification
Samples were quantified using the Quantifiler™ Duo Kit (Thermo Fisher Scientific, South San Francisco, CA) according to the manufacturer (2) on a 7500 real-time PCR instrument (Thermo Fisher Scientific).

Amplification
Select samples were amplified using the Investigator 24plex QS Kit (QIAGEN) according to the manufacturer (3) on a ProFlex™ thermal cycler (Thermo Fisher Scientific).

RESULTS AND DISCUSSION

Comparison of all semen and non-semen samples

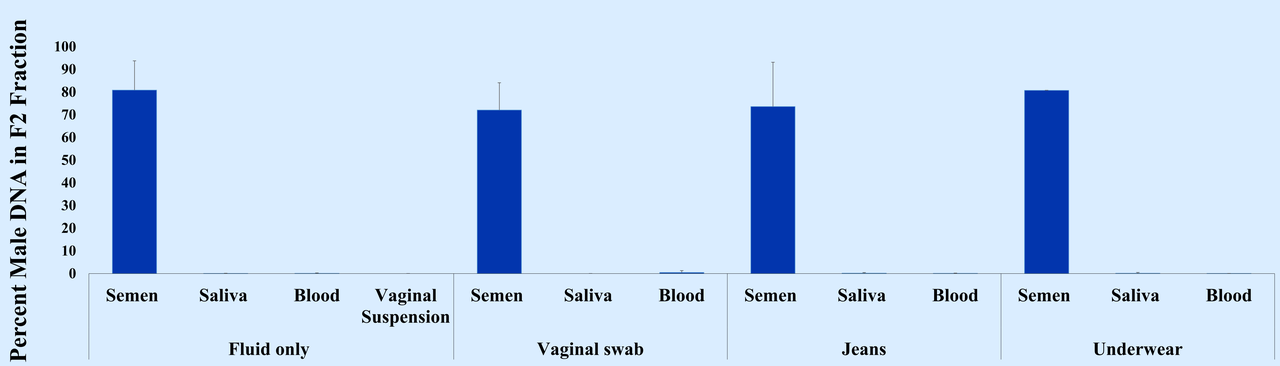

Figure 1. Graph showing the percentage of male DNA present in the F1 fraction for various sample types. Male blood and saliva was used. N=9 for each body fluid.

- Higher percentages of male DNA observed in the F2 fraction for all semen samples compared to non-semen, male samples (Figure 1).
- Highest average percentage of male DNA found in the F2 fraction for semen samples was 80.8% while the lowest average percentage for semen samples was 72.1% (Figure 1).
- Generally, less than 1% of male DNA was found in the F2 fraction for all non-semen samples (Figure 1).
- Highest average amount of male DNA detected in the F2 fraction for non-semen samples was 0.5% seen in the male blood deposited onto vaginal swab (Figure 1).

Comparison of total human and male DNA ratios

Table 1. Enrichment results for F1 and F2 fraction of male body fluids deposited on vaginal swabs.

Vaginal Swab Sample	Dilution	Human:Male F1	Total DNA (ng)	Human:Male F2	Total DNA (ng)
Semen (Donor 1) (n=3)	Neat	440:1	55.21	1:1	1.24
	1:10	86:1	67.68	1:1	1.12
	1:100	1942:1	53.04	1.5:1	0.3
Male Blood (n=3)	Neat	9:1	56.39	6:1	0.13
	1:10	54:1	53.64	33:1	0.15
	1:100	850:1	50.77	202:1	0.15
Male Saliva (n=3)	Neat	1581:1	49.92	NaN*	0.03
	1:10	5324:1	44.91	NaN*	0.01
	1:100	NaN*	46.34	NaN*	0

*NaN=not a number, no male DNA recovered

- Human:Male DNA ratio in favor of female contributor in F1 fraction for all samples (Table 1).
- ~1:1 Human:Male ratio in the F2 fraction for semen sample; male DNA enriched (Table 1).

Table 2. Percentage of profile contributor calculated in STRmix

Vaginal Swab Sample	Dilution	F1		F2	
		Percent contributor 1 (Female)	Percent contributor 2 (Male)	Percent contributor 1 (Female)	Percent contributor 2 (Male)
Semen (Donor 1) (n=1)	Neat	98.99	1.01	11.96	88.04
	1:100	99.67	0.33	59.10	40.90
Male Blood (n=1)	Neat	89.83	10.17	91.24	8.76
	1:100	99.61	0.39	74.91	25.09
Male Saliva (n=1)	Neat	98.91	1.09	97.98	2.02
	1:100	98.75	1.25	99.48	0.52

- Percent contribution of DNA in favor of female contributor (>80%) in F1 fraction for all samples (Table 2).
- Male DNA enriched in the F2 fraction for semen sample (Table 2).

MATERIALS AND METHODS

Capillary Electrophoresis and Interpretation
Amplified products were separated and detected by capillary electrophoresis on a 3500 Genetic Analyzer (Thermo Fisher Scientific) using POP-4 polymer on a 36 cm capillary. DNA profiles were analyzed using ArmedXpert version 3.0.8.34 (NicheVision Forensics, Akron, OH, USA) software applying a 100 RFU analytical threshold and using the Investigator 24plex QS Kit manufacturer stutter thresholds.

Data Analysis
Percentage of male DNA present in the F2 fraction:
Equation 1.
(Male DNA F2)/(Male DNA F1+Male DNA F2) x 100%

Percentage of male DNA present in the F1 fraction
Equation 2.
1-(Male DNA F2)/(Male DNA F1+Male DNA F2) x 100%

STR profiling success was assessed based on the percentage of reportable alleles, average peak height, heterozygote peak height ratio (PHR) and the relative presence of a mixture.

STRmix™ (ESR, Auckland, New Zealand) software was used to deconvolute female-male mixed profiles. Ratios of total human and male DNA between the F1 and F2 fractions were calculated for each donor. Ratios calculated using quantification data were compared to ratios of the STRmix™ output.

CONCLUSIONS

- Male DNA from sperm is more prevalent in the F2 fraction following differential extraction.
- Less than 1% of male DNA present in the F2 fraction for non-sperm samples.
- Quantification data may be used to infer presence of sperm in cases of poor serological testing.
- Examination of DNA fractionation may aid in weighing activity level propositions.

REFERENCES

1. NYC Office of Chief Medical Examiner. Forensic biology protocols for forensic str analysis-differential extraction from semen stains or swabs utilizing the qiagen qiacube and qiagen ez1 instruments.
2. ThermoFisher Scientific. User Guide: QuantifilerTM Duo DNA Quantification Kit. 2018.
3. QIAGEN®. Investigator® 24plex QS Handbook. 2021.